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"Rapid Determination of Microbial Numbers in Foods"

FINAL TECHNICAL REPORT

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U. S. Army Research Office

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18. SUPPLEMENTARY NOTES

The findings in this report are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

SEE REVERSE SIDE (page 3)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The primary objective of this research was to study the reactivity of meat-borne gram negative bacteria with the Limulus amoebocyte lysate (LAL) reagent relative to the use of LAL as the basis of a 1-hour method for determining the overall safety or quality of fresh meats, and specifically to determine the numbers of microorganisms in such products in 1 hour. Preparatory to the development of the 1-hour test, the various parameters that affect

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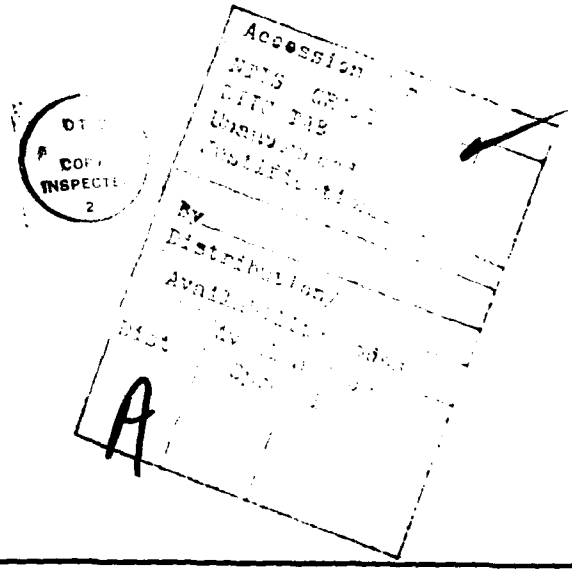
LAL reactivity of foodborne gram negative bacteria were investigated. This phase of the project involved the development of selective media for both gram negative and gram positive bacteria, the relative incidence of yeasts and molds in the products studied relative to bacterial numbers, incidence and types of lactic acid bacteria, and the effect of members of the flora on gram negative endotoxins. In the development of a selective medium for gram positive bacteria, one compound (diacetyl) tested for activity against gram negative bacteria and fungi proved to be of much greater interest as an antimicrobial agent for food use, and research on this compound was pursued at the expense of the further development of the medium for gram positive bacteria. Further research with this and similar compounds may lead to the establishment of a new class of antimicrobial agents.

19. Key words.

Microorganisms in foods
Limulus amoebocyte lysate test
Gram negative bacteria, meat-borne
Yeasts, in ground beef
Lactic acid bacteria in ground meats
Meats, microbiology of
Lipopolysaccharides, determination in meats
Endotoxins (see lipopolysaccharides)
Diacetyl, antimicrobial activity of
2,3-butanedione (see diacetyl)
Rapid methods, for bacteria in meats



SUMMARY OF FINDINGS

1. In regards to the best method for extracting both microorganisms and endotoxins (lipopolysaccharides, LPS) from meats, homogenizing with a stomacher, shaking by hand, and blending with Waring blender were compared and the stomacher method was found to be the method of choice (Jav and Margitic, 1979).

2. A strong correlation of LPS in fresh ground meats with viable microbial numbers was established --- meats with low quantities of LPS by LAL also have low viable counts (Jav et al., 1979).

3. In regards to the utility of using 2-fold sterial dilutions versus the most probable number (MPN) method for determining LPS in meats, it was revealed that the two methods gave essentially similar results (Seiter and Jav, 1980).

4. When it was noted that many of the meat-borne gram negative bacteria did not grow upon primary plating onto media normally used for gram negative enteric bacteria, a medium was developed which has proven to be much more effective for growing meat-borne psychrotrophic gram negative bacteria (Cyzeska et al., 1981).

5. In an effort to determine the utility of using ratio values of yeasts and/or molds relative to bacteria in meats in order to make 1-h estimates of viable numbers, the incidence of yeasts in fresh ground beef was compared to that of bacteria. Although the ratio values proved to be rather variable, this study is the first one of any detail on the incidence of yeasts in fresh ground beef (Jav and Margitic, 1981). The generic and species identification of yeasts from ground beef is likewise the first such report (Hsieh and Jav, 1982).

6. The incidence and types of lactic acid bacteria in fresh meats has not been reported. Preliminary evidence has been obtained indicating that some of these organisms (streptococci) may be effective in degrading LPS. A manuscript on the taxonomic aspects is in preparation (Jay et al., 1982a).

7. The basis and operation of the 1-h test for estimating the numbers of total viable bacteria in fresh ground beef was developed (Jav, 1981;1982b).

8. The relative sensitivity of meat-borne psychrotrophic bacteria to LAL was studied. Previous reports in the literature deal only with gram negative bacteria of clinical importance (Shereda and Jay, 1979). This aspect of the project is the subject of an M. S. thesis (A. L. Shereda) and a journal paper is anticipated later in 1982.

9. The relative effect of pH of medium, temperature of incubation, nutrient composition of medium, and age of culture on the reactivity of LPS with LAL was studied. A manuscript on this aspect of the project has not yet been completed but is anticipated by late summer of 1982 (Jay, Beat, and Seiter, 1983).

10. In the course of studies on the development of a medium selective for all gram positive bacteria, diacetyl (2,3-butanedione) was uncovered as a very unique antimicrobial compound in that it is more effective against gram negative bacteria and fungi than gram positive bacteria. Since it is a GRAS substance, this finding should prove to be of high interest to microbiologists interested in antimicrobial agents, and to those interested in inhibitors in the food industry. Since diacetyl is a vicinal diketone, this aspect of the project leads one to believe that a new class of microbial inhibitors based upon this property may be possible (Jay, 1982c,d,e).

LIST OF PUBLICATIONS

A. M. S. Thesis.

Cyzeska, Frank J. 1979. A new selective medium for the isolation and enumeration of psychrotrophic gram negative bacteria. Wayne State U.

B. Abstracts.

1. Shereda, A. L. and J. M. Jay. 1979. The relative sensitivity to Limulus amoebocyte lysate (LAL) of meat-borne psychrotrophic gram negative bacteria. Bacteriol. Proc., P-7.

2. Jay, J. M. 1982c. Effect of diacetyl on foodborne micro organisms. Proc., Inst. Food Technologists.

C. Book chapter.

Jay, J. M. 1982b. Analysis of food products for microorganisms or their products by nonculture methods, pp. IN: Food Analysis: Principles and Techniques, J. R. Whitaker and D. W. Gruenwedel, eds. (Marcel Dekker: N.Y.). In press. NOTE: Basis of the 1-h test for microbial numbers employing LAL data is explained along with other nonproject methods.

D. Journal articles.

1. Jay, J. M. and S. Margitic. 1979. Comparison of homogenizing, shaking, and blending on the recovery of microorganisms and endotoxins from fresh and frozen ground beef as assessed by plate counts and the Limulus amoebocyte lysate test. Appl. Environ. Microbiol. 38: 879-884.

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9. Hsieh, D. Y. and J. M. Jay. 1983. Characterization and identification of yeasts from fresh and spoiled ground beef. J. Food Sci. (submitted, 6/82).

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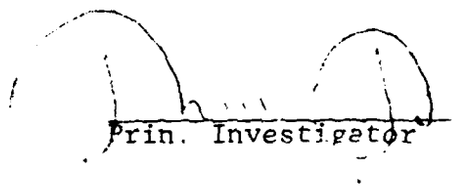
11. Jay, J. M. and C. Hasegawa. 1983. The effect of diacetyl on the spoilage of fresh refrigerated ground beef. J. Food Sci. (In preparation).

LIST OF PERSONNEL

Name	Role in project	Dates on project
Margitic, Slavoljub, M.S.	Research Assistant	11/1/78 - 10/31/79.
Cyzeska, Frank J.	Grad. Res. Asst.	1/1/79 - 9/12/79.
Hsieh, Dick Y.	Student Assistant	11/1/78 - 3/21/80.
Seiter, Julie A., Ph.D.	Research Associate	11/1/79 - 10/31/80.
Smith, Lorraine V.	Student Assistant	1/17/80 - 4/29/80.
Marks, Stephen D.	Student Assistant	4/2/80 - 8/20/80.
Beat, David A., Ph.D.	Research Associate	11/1/80 - 10/30/81.
Belton, Margaret	Student Assistant	12/8/80 - 4/30/82.
Duthie, Wendy E.	Student Assistant	4/1/81 - 7/29/81.
Hasegawa, Cynthia	Student Assistant	1/1/82 - 4/30/82.
Boisvert, William E.	Student Assistant	1/1/82 - 4/30/82.
Jay, J. M.	Principal Invest.	11/1/78 - 4/30/82.



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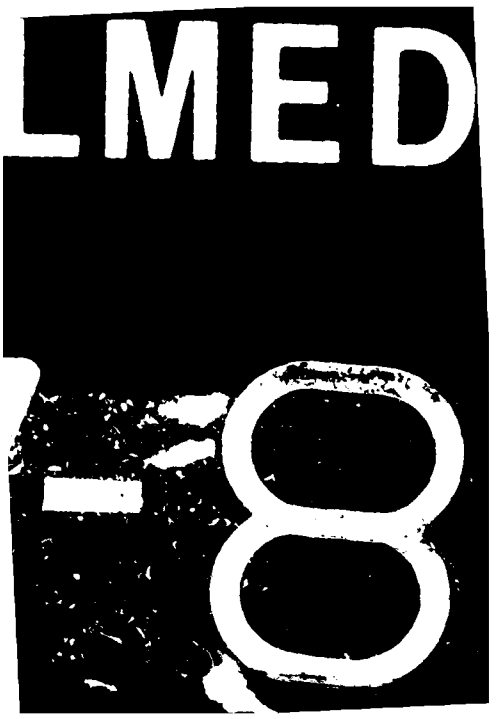
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